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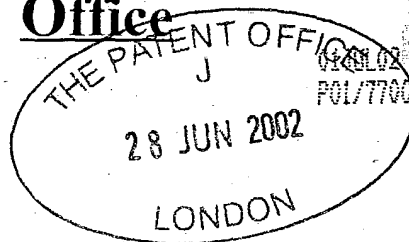
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Dated

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POL/7700 0.00-0215023.3

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet, from the Patent Office to help you fill in this form)

1. Your reference JMCV0317.GB

2. Patent application number 0215023.3 28 JUN 2002

3. 1. Name, address and postcode of the or of each applicant (underline all surnames) Bristol-Myers Squibb Company
345 Park Avenue
New York
NY 10154
United States of America

Patents ADP number (if you know it)

4448882001

If the applicant is a corporate body, give the country/state of its incorporation New York, United States of America

4. Title of the invention Wound Dressing

5. Name of your agent (if you have one) Barker Brettell
"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode) 10-12 Priests Bridge
LONDON
SW15 5JE

Patents ADP number (if you know it) 7442494003

6.	Country	Priority application number (if you know it)	Date of Filing (day/month/year)
If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number			

7.	Number of earlier application	Date of filing (day/month/year)
If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application		


8. Is a statement of inventorship and of right to grant of a patent required in support of this request (Answer 'Yes' if:
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d)) Yes

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Do not count copies of the same document
Continuation sheets of this form

Description 9

Claim(s) 1

Abstract

Drawing(s) 2 

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

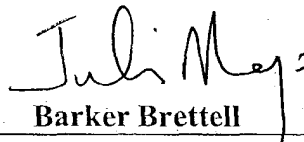
Request for preliminary examination 1
(*Patents Form 9/77*)

Request for substantive examination
(*Patents Form 10/77*)

Any other documents
(*please specify*)

11. I/We request the grant of a patent on the basis of this application.

Signature


Barker Brettell

Date

28 June 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Julie Mays

Tel: 020 8392 2234

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Patents Form 1/77

Wound Dressing

The present invention relates to wound dressings having antibacterial, antiviral and/or antifungal activity, to a method of producing such dressings and the use of such dressings in the treatment of wounds.

With the rise in antimicrobial resistance and a general call to reduce the use of antibiotics, silver is gaining increasing popularity as an effective antimicrobial agent. The advantage of using silver as a bacteriostatic agent is that there is no formation of bacterial tolerance. This is in contrast for instance to many antibiotics. A major drawback when using ionic or metallic silver for bacteriostatic purposes is however the lack of control over release of the silver ions from the delivery vehicle.

In the past it has been known to deliver silver ions by the use of a simple solution of silver nitrate. It is also known to deliver silver by the use of a complex with sulfadiazine. Silver sulfadiazine is used extensively in the treatment of wounds, and particularly burns, and is incorporated in a cream base and sold under the trademark Flamazine. As the silver is present in such products as a complex, its solubility in wound fluid is low and hence the quantity of active silver present is also low.

By contrast, if the delivery vehicle for the silver does not limit the amount of ionic silver entering the wound fluid, too high a concentration of silver ions is dumped into the wound fluid and the silver may deposit as metallic silver or dark coloured salts on the wound and skin. This can result in staining of the tissues. Such staining has been reported to give potentially permanent pigmentation of the skin, so called argyria. It is also known to deliver silver to the wound by fragmentation of metallic silver particles from a dressing. Such dressings are sold under the trademark Acticoat. These dressings may also give rise to staining of the

wound, surrounding skin and other materials such as clothes or bed linen by deposition of metallic silver.

There thus exists a need for a delivery vehicle for silver ions which
5 controls the release of silver ions to the wound fluid so that staining is minimised but an effective concentration is maintained to give the desired antimicrobial activity.

A further disadvantage of dose dumping of silver ions into the wound
10 fluid is that the dressing must necessarily be frequently changed to maintain a constant presence of antimicrobial agent and prevent infection. This is also true of treatments which deliver very low concentrations of silver ions such as silver sulfadiazine. The repeated changing of dressings on for instance burns patients causes pain to the patient and disturbs the
15 healing process. It may be necessary for burn wounds to be dressed for three weeks or more. There thus exists a need for a wound dressing with sustained release of silver ions which maintains an effective concentration over a prolonged wear time.

20 WO/02 43743A to Bristol-Myers Squibb describes the preparation of a material which contains one or more hydrophilic, amphoteric or anionic polymers, where the material has antimicrobial activity. The material is prepared by preparing a solution comprising an organic solvent and a source of silver, subjecting the polymer to the solution to incorporate a
25 desired silver concentration into said polymer, and subjecting the polymer during or after this step to one or more agents that bind the silver to the polymer and render it photostable upon drying. The polymer is, for example, a polysaccharide and, particularly, a carboxymethylcellulose or an alginate or a mixture thereof. WO/02 43743A is not concerned with
30 skin staining caused by silver-containing wound dressings.

We have now found that wound dressings can be prepared which give a controlled, sustained release of silver ions into wound fluid without staining the underlying tissue.

5 Accordingly, the invention provides for the use of an effective amount of silver in the manufacture of a wound dressing comprising an anionic polymer, which dressing, when applied to the wound, gives a controlled release of ionic silver into the wound fluid for the prevention of staining of the underlying tissue.

10

The wound dressing for use in the present invention comprises an amphoteric, hydrophilic, anionic polymer such as polysaccharides or modified polysaccharides, polyvinylpyrrolidone, polyvinyl alcohols, polyvinyl ethers, polyurethanes, polyacrylates, polyacrylamides, collagen, 15 gelatin or mixtures thereof. In preferred embodiments, the polymers contain carboxymethylcellulose (CMC) such as sodium CMC. In one embodiment the polymer can be a polysaccharide comprising a carboxymethylcellulose or alginate or a mixture of carboxymethylcellulose and alginate. In other embodiments, the polymers 20 contain gel-forming fibres comprising sodium CMC and which can be incorporated into wound dressings such as Aquacel (ConvaTec, Skillman, NJ). The polar or ionic nature of the polymer means that the binding of positively charged silver ions (cations) is facilitated.

25 We have found that a desired final concentration of silver in the dry wound dressing is between about 0.1% and 20% by weight, for example. Preferably between 0.1% to 10% by weight and more preferably between 0.5% and 5% by weight of the dressing. Such concentrations can be achieved by the preparation method described in WO/02 43743A.

30

Whilst not wishing to be bound by theory, the inventors hereof believe that the concentration of silver ions in the wound fluid is controlled by the rate of dissociation from and diffusion through the dressing of the silver ions and the solubility of silver chloride formed in the wound fluid.

- 5 The solubility of silver chloride in wound fluid is believed to be about 1 ppm. It is believed that the amount of silver ions released is controlled by the volume of wound fluid and by the formation of a cohesive gel which restricts migration of silver ions out of the dressing. The dissociation of silver ions from the dressing into the wound fluid retained
10 within the dressing tends the concentration of silver ions towards a constant of less than about 1 ppm regardless of the volume of wound fluid. In the presence of large amounts of wound fluid and high concentrations of micro-organisms, more silver ions become available. This allows the silver ions to be available and active only when they are
15 required and prevents the deposition of metallic silver or silver compounds which lead to staining. It also means that as the rate of exudate production reduces so does the rate of dissociation of silver, meaning that the weight percent of silver in the dressing is less than would be expected to maintain effective levels of silver over the wear
20 time of the dressing.

The silver is preferably bound to the anionic, amphoteric, hydrophilic polymer by the use of a binding agent for photostabilization. Suitable agents include ammonia and chlorides.

25

- The dressing is preferably in the form of a fibrous mat of the polymer but may be in the form of woven fabric or a powder or distributed within a matrix of a hydrocolloid or acrylate adhesive. The dressing can be used as part of a larger dressing or a layer in a multi-layered dressing and need
30 not be in direct contact with the wound.

The invention is illustrated by the following examples.

Example 1

5 Analysis of Silver-containing Dressings and treatments

A dressing for use in the invention and various commercial silver-containing dressings were analysed for various properties. The data is presented in the table below.

10

AQUACEL-Ag samples were prepared according to the method of WO 02 43743A.

Weight per unit area was determined by weighing a complete dressing and
15 dividing by its measured dimensions.

Loss on drying was performed gravimetrically. A minimum of 1g of sample (or a whole dressing where possible) was placed in a tared dish, weighed, heated at $105^{\circ} \pm 3^{\circ}\text{C}$ for 6 hours, allowed to cool in a
20 desiccator and then reweighed.

Silver assay was performed using atomic absorption spectrophotometry (AAS) on a wet acid digestion. If insoluble matter was present after the digestion procedure was completed it was removed by filtration prior to
25 assay.

Dissolution experiments were carried out using a standard tablet dissolution apparatus. Approximately 3g of each test dressing were sealed into a pre-washed and hydrated cellulose dialysis membrane bag
30 (Sigma D-9402). This was then loosely attached to the stirring paddle of the dissolution apparatus using plastic cable ties. The sample was lowered

into the receiving vessel containing 300ml of dissolution medium at 37°C. The stirring rate was set at 60rpm. The temperature was maintained at 37°C and the dissolution medium sampled (10ml) at regular intervals. The sample volume was kept at 300ml by regularly topping up with the dissolution medium. The dissolution media used were (i) Normal saline (0.9%w/v NaCl(aq)) and (ii) purified water. The free silver content of the solutions was determined directly by AAS.

Normal saline was chosen to be a simple model of wound exudate in which the naturally occurring high chloride concentration would compete for available ionic silver, attempting to precipitate it from solution as insoluble silver chloride. Water was used as an alternative medium to observe the chloride free rate of silver delivery.

Silver Assay Data

Sample	Batch No	Dressing Weight/Area as received (mg/cm ²)	LOD 6 hrs 105°C (%w/w)	Silver as received (% w/w)	Silver as received (mg/cm ²)	Silver on dry weight (% w/w)
AQUACEL-Ag	A4592	9.53	9.94	1.153	0.110	1.280
Acticoat Burn	001103A-02	9.63	2.63	10.088	0.972	10.361
Acticoat 7	010320A-03	17.6	2.93	8.414	1.484	8.668
Acticoat Absorbent (Alginate)	010524A-04	14.5	14.76	8.492	1.230	9.967
Acticoat Moisture Control	010316A-01	64.4	2.39	1.992	1.282	2.041
Actisorb Silver 220	0117-01	16.9	13.60	0.069	0.012	0.079
Avance Foam	1036163	61.4	1.50	0.006	0.004	0.006
Flamazine Cream	11013	390 (Note 1)	72.36	0.298	1.16 (Note 2)	N/A

Notes

- 1) Flamazine cream has a density of 0.975g/cm³ (BB867 page 6)
- 2) Assuming a 4mm layer is applied per treatment
- 3) LOD stands for loss on drying

- The Acticoat 7 and Burn products are all based polyethylene mesh coated with silver which is sprayed on. Acticoat absorbent is an alginate based product again with sprayed on silver. Acticoat moisture control is a foam product coated with silver. Avance is a foam with a zirconium ion exchange material distributed within it. Actisorb Silver 220 is a nylon bag containing a silver impregnated charcoal cloth.

Silver Dissolution Rate into Water (Total Volume 300cm³, stirred, 37°C).

10 Parts per million silver in solution (ppm) ($\mu\text{g/ml}$)

Sample	Sample Mass (g)	Assay Time (hours)						
		1.5	18	98	120	144	168	187
AQUACEL-Ag A4591	3.2843	0.0117	-	0.623	0.712	0.907	0.883	0.922
AQUACEL-Ag A4592	3.0435	0.0439	0.12	0.44	0.67	1.16	0.96	0.688
Acticoat Burn	3.1367	4.56	-	25.00	31.00	28.90	30.70	22.6
Acticoat 7	3.2578	7.17	-	24.90	34.60	32.30	33.50	26.3
Acticoat Absorbent (Alginate)	3.2649	12.8	-	26.2	30.5	30.8	30.9	26.6
Acticoat Moisture Control (Foam)	3.3137	4.06	-	5.3	9.58	8.73	7.73	5.40
Actisorb Silver 220	3.3166	0	-	0	0.02	0.03	0	0.0617
Avance Foam	2.9352	0	-	0.09	0.05	0.06	0	0.0897
Flamazine	2.6419	0.0067	-	0.294	0.363	0.349	0.353	0.423
Control (AQUACEL)	3.2100	0	-	0	0	0	0	0.0093

- These results indicate that the silver metal-based Acticoat range of dressings would deliver high levels of solubilised silver rapidly to a moist wound where it will be precipitated as silver chloride. This dose dump effect, together with any metallic silver or silver oxide (Acticoat) will be deposited in the wound bed where it may cause cytotoxic effects and skin staining.

- 20 Avance contains only trace amounts of silver. In the dissolution experiments this was found to be readily released. In a moderately

exuding wound one would expect this quantity of silver to be rapidly depleted and the dressing to become ineffective in the control of microbes.

- 5 Actisorb Silver 220 delivers solubilised silver very sparingly and its is predicted that although microbial growth may be retarded within the charcoal cloth, this would have very little effect on reducing the bioburden of a wound.
- 10 The mechanism by which Flamazine works is not clearly demonstrated by these experiments. Soluble silver availability is relatively low on a weight/weight basis, but repeated application of a large dose (weight/area) as indicated in the instructions for use will increase availability as will any surface active and lypophilic effects of the base
- 15 ointment. Its action will also be complemented by the antimicrobial activity of the sulphadiazine component.

Example 2

20 Whole Skin Staining

- Skin staining studies were carried out using an adaptation of a Franz type horizontal glass diffusion cell as described in Dugard et al, 1984. Whole human skin and wound tissue samples (1 cm² discs) were punched out
- 25 using an Osborne arch punch and placed epidermal side uppermost on the receptor chamber. The donor chamber was then gently placed on top of the receptor chamber. The whole cell was clamped together and placed into a water bath maintained at 32°C ± 1°C. The underlying whole skin was bathed in saline.

Individual silver-containing wound dressings (0.64-1 cm²) were cut out and placed onto the centre of a piece of human whole skin. 200 μ l of saline was placed into the donor chamber to hydrate each dressing to mimic a moderately exuding wound. Approximately 5-10mg of Flamazine cream was placed onto the skin and gently smoothed over the exposed skin surface by means of a small spatula. Silver nitrate solution (200 μ l, 0.5%) was used as the positive control and water was used as the negative control. Each cell was left for 24 hours and images were taken using a Polaroid digital microscope camera.

10

The results are presented in Figure 1. It can be seen that significant silver staining is obtained with silver nitrate, Acticoat Burn and Acticoat 7. There is minimal staining with Flamazine cream. Aquacel Ag was prepared as described in WO 02 43743A. As can be seen no skin staining was obtained by the use of Aquacel Ag according to the invention.

15

Example 3

20 Wound Tissue Staining Studies

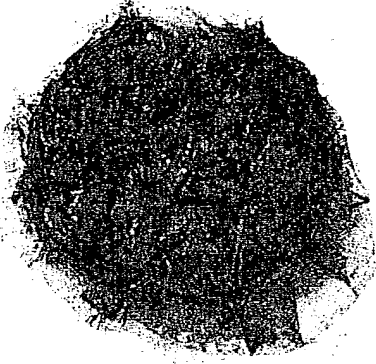
In these studies, a dressing for use in the invention, Aquacel-Ag, prepared according to WO 02 43743A, and Acticoat 7 were applied to human ulcer tissue and left in contact with the wound for 24 hours.

25 Saline was used as a control. The results are shown in Figure 2. The results show that no staining was obtained with use of the dressing according to the invention.

WHAT IS CLAIMED IS:

1. Use of an effective amount of silver in the manufacture of a wound dressing comprising an anionic, amphoteric or hydrophilic polymer,
5 which dressing, when applied to a wound site, gives a controlled release of ionic silver into the wound fluid for the prevention of staining of the underlying tissue.
2. Use of an effective amount of silver in the manufacture of a wound
10 dressing comprising an anionic, amphoteric or hydrophilic polymer, which, when in contact with wound exudate, gives a controlled release of ionic silver into the exudate for the maintenance of an effective antimicrobial activity within the dressing throughout the wear time of the dressing.
- 15 3. Use as claimed in any preceding claim wherein the anionic, amphoteric or hydrophilic polymer is selected from the group of polysaccharides or modified polysaccharides, polyvinylpyrrolidone, polyvinyl alcohols, polyvinyl ethers, polyurethanes, polyacrylates,
20 polyacrylamides, collagen, gelatin or mixtures thereof.
4. Use as claimed in any preceding claim wherein the dressing is in the form of fibres, or a powder or distributed within a matrix of an adhesive.
- 25 5. Use as claimed in any preceding claim wherein the dressing comprises from 0.1 to 20% by weight of silver.
6. Use as claimed in any preceding claim wherein the release of anionic
30 silver into water is less than 1 ppm.

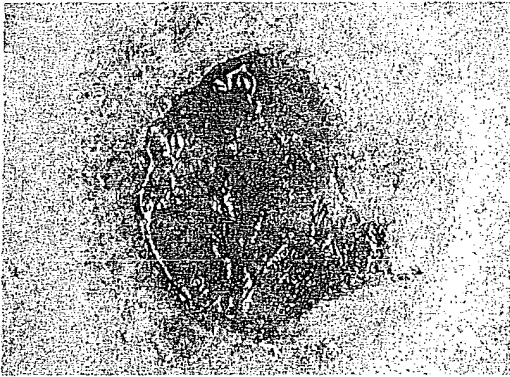
1/2



A. Silver nitrate (positive control)



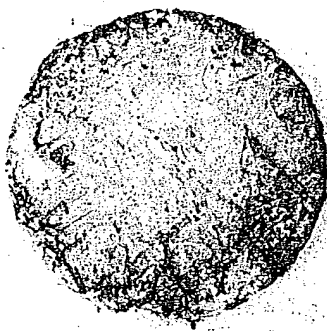
B. Saline (negative control)



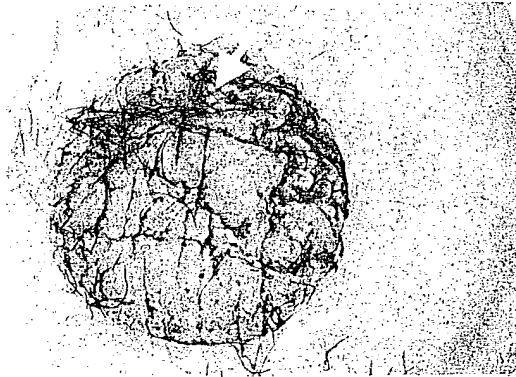
C. Flamazine



D. AQUACEL-Ag



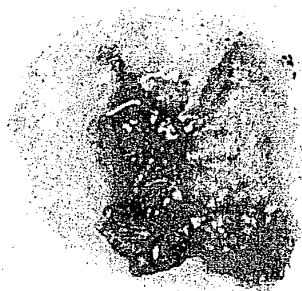
E. Acticoat 7



F. Acticoat Burn

Figure-1

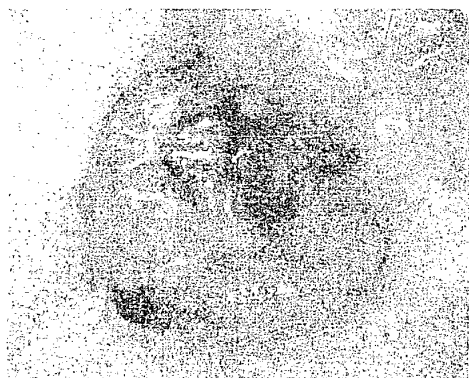




Acticoat™ 7 application to wound tissue



AQUACEL-Ag application to wound tissue



Saline control

Figure 2



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